Antimicrobial resistance and clonality in Acinetobacter baumannii

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CHAPTER 7

Summary

SUMMARY

Acinetobacter baumannii is an important nosocomial pathogen which gives rise to colonization and infection of patients, in particular in intensive care units. The strains involved are frequently resistant to multiple antimicrobial agents, leaving limited therapeutic options, and these strains can spread among patients. In 1996, it was shown that two groups (termed European (EU) clones I and II) of genotypically highly similar *A. baumannii* strains prevailed among isolates from outbreaks in geographically distant hospitals of north-western Europe. This observation was corroborated by the study published in 1999 that showed that strains related to EU clones I and II predominated among clinical isolates of *A. baumannii* in the Czech Republic. Both studies also showed that the strains belonging or related to EU clones I and II were more resistant to antimicrobial agents than other strains and it has been suggested that the association of multi-drug resistance and clonality might have played a role in the progressive increase in *Acinetobacter* resistance. These findings and considerations gave rise to the attempt to gain insight into the epidemiology and molecular basis of multidrug resistance of *A. baumannii* at the population level. To this aim, a descriptive and comparative approach was used to analyse a large number of properties in well-defined collections of strains in order to assess the relationship between multidrug resistance and the population structure of *A. baumannii*. The studied organisms consisted mainly of clinical isolates collected since 1991 from hospitalized patients in the Czech Republic and of isolates from various other European countries enrolled in the collection of the Leiden University Medical Centre over the recent two decades.

In the first study, we investigated the genotypic relationship between Czech *A. baumannii* strains previously assigned to the so-called groups A and B and the north-western EU clones I and II using AFLP fingerprinting and ribotyping. The study collection included 70 multidrug resistant (MDR) and 15 susceptible strains from 1991-2001 from the Czech Republic and reference strains of EU clones I and II. The results confirmed that the two predominant groups A and B observed among the MDR Czech *A. baumannii* strains from the 1990s were genomically congruent with clones I and II, respectively. In the next study, we investigated the diversity of the genes encoding aminoglycoside-modifying enzymes and their association with class 1 integrons in the EU clones including also the newly described clone III. We found that, whereas the clone III strains were relatively homogeneous both in resistance genes and integrons, clone I and II showed a remarkable intraclonal diversity of these properties, with no clear-cut difference between the two clones. Yet, within the Czech clone I and II strains, the diversity of resistance genes and integron structures was limited as compared to those from other countries. These findings suggest the occurrence of local pools of resistance genes and the possibility of horizontal transfer of resistance genes between the two clones.

In the third study, the question of the clinical-epidemiological relevance of the recently discovered nonspecific efflux pump AdeABC in *A. baumannii* was addressed. A total of

116 strains from 16 European countries isolated over a period of 23 years were investigated for the presence of genes associated with the efflux system. Furthermore, the diversity of the strains (clonal relatedness or unrelatedness) was assessed by AFLP fingerprinting. Results showed that the AdeABC genes were present in most *A. baumannii* strains including fully susceptible strains, and that overexpression of this system is likely to be a common property of MDR strains. The purpose of the last study was to analyse the emergence of carbapenem resistance among *Acinetobacter* hospital strains in the Czech Republic, which has been noted from the early 2000s onwards. To this aim, *Acinetobacter* clinical isolates were collected prospectively from multiple ICUs in 2005-2006 and were analysed for their genomic types and for resistance determinants. The results showed that the emergence of carbapenem resistance was associated with the spread of *A. baumannii* strains of a subclone of EU clone II. An impressive variation in resistance determinants in this group of highly related strains was observed. Finally, in chapter 6 of the thesis the overall results of the studies are discussed within the context of the most recent developments.

The present thesis provides an important contribution to the concept that the increasing resistance to multiple antibiotics in *A. baumannii* is significantly associated with the occurrence of a limited number of widespread groups of genetically closely related strains (clonal lineages). Among them, the so-called EU clone I and II seem to have prevailed among MDR *Acinetobacter* strains in some European countries since the early 1980s. Whereas clone I dominated over clone II among the strains isolated in the 1980s, more recent studies indicate a growing importance of clone II. Our results as well as other studies have shown that resistance to carbapenems, the most important group of antibiotics against MDR acinetobacters, can be associated with some geographic subclones of EU clone II, which suggests that this clone plays an important role in the development and spread of carbapenem resistance. Recent studies including ours have identified a number of resistance mechanisms in *A. baumannii* and have shown that multiple mechanisms can be present in particular strains. It has become clear that both activation of intrinsic resistance mechanisms and horizontal acquisition of resistance genes play a role in the evolution of antibiotic resistance of this organism. In addition, very recent studies have reported unique genetic structures termed resistance islands which harbour acquired resistance genes and can be responsible for a striking variation in resistance genotypes and phenotypes in clonally and epidemiologically related isolates. Thus, recent findings including the observations of the present thesis are bringing up new, more basic, questions and opening a window to experimental studies that can lead to a comprehensive understanding of factors which contribute to the ability of *A. baumannii* to develop resistance to all clinically relevant agents.